

PATHOLOGICAL ANATOMY OF THE INJECTED TRUNKS OF CHESTNUT TREES.

(PLATES XV-XVIII.)

BY CAROLINE RUMBOLD, PH.D., DR. ŒC. PUBL.

(Read March 3, 1916.)

While working on tree injection in connection with the chestnut-tree blight, a large number of Paragon chestnut trees (orchard trees) were made the subjects of experimentation by introducing into their trunks different substances in solutions of varying dilution. The method used in making the injections has been described in *Phytopathology* (1).

The injections were made to discover the effect of the chemicals on the chestnut trees and in turn on the parasitic fungus *Endothia parasitica* (Mur.) A. & A., the cause of the chestnut-tree blight. The reason for undertaking these experiments is mentioned, because the processes devised for this investigation not discussed in this paper, have rendered the phase of results here presented, somewhat uneven and unfinished. Further study on this subject is in progress.

The chemicals used in the injections were about 50 in number: hydrocarbons, metals and alkali metals. So far an examination of the trunks and branches of injected trees shows that their reactions to the different chemicals were alike in kind but varied in intensity. The effect of the chemicals on the leaves of the trees differed, but this will not be discussed at this time.

The injected solution passed through the vessels of the youngest and the one year old rings of wood. There were exceptions to this rule, which will be explained later.

The reaction in the trunk and branches of the tree varied with the distance from the point of injection. The affected region extended up and down the trunk in a line whose width usually was but little more than the injection hole. The cells through which the

solution passed acted like a blotter, with the result that the farther from the point of injection, the more dilute was the solution and the smaller the injected stream. Correlated with this, the tree tissues appeared more normal as the distance from a point of injection increased and the area of disturbance decreased. Occasionally all stages of reaction to an injection could be seen in a tree: death—at the point of injection—retarded growth, stimulated growth and no reaction.

The results of the injections, to which particular attention is called here, are of interest from a histological standpoint.

1. A strong inhibitory effect on the growth of the cambium layer was noticed; so strong that as a tissue it often disappeared. The cambium cells changed into xylem.

2. There was an irregular formation of the new year ring of wood. During its formation isolated groups of xylem cells appeared in the midst of the phloëm.

3. Phloëm cells were converted into xylem by cell division followed by lignification, or the cell walls were lignified without cell division.

4. All of the cells of the phloëm region were capable of change with the exception of the stone cells, the bast-fibers and their accompanying cells containing crystals.

5. There was a production of wound tissue showing various degrees of abnormality. The wound tissue was abnormal in that its position was reversed from the one in which it is customarily seen, and frequently the cells composing it had unusual shapes.

6. Cork formed prematurely. It was apparently correlated with the irregular growth of xylem in the phloëm region.

Another region of response to the injections was the xylem, evidenced by an increased formation of thylloses and a thickening of cell walls. This response, while pathological, was one which was expected. For this reason no emphasis is placed upon it in this discussion, but it will be referred to in connection with the paths of the injected solutions.

These points can be elucidated best by a view of some sections cut from injected trees. They have been selected from many, as they illustrated the points emphasized in this paper.

OBSERVATIONS.

The Path of the Injected Solutions in the Trees.

As stated before, the path of the solutions usually was through the vessels of the youngest ring of wood (Figs. 2, 5, 10, and 11). It sometimes happened that the stream was shifted from these vessels. Fig. 1 shows such a case. This section was cut from a tree which had been injected with methylene blue. The stain had colored the passages taken by it. The solution of methylene blue was not toxic, but it was stimulating enough to cause the formation of thylloses, which finally plugged the vessels through which the stain passed. The main stream then passed through the vessels of the older year ring. The amount of such shifting appeared to vary with the toxicity of the injected chemical, for killing solutions never changed their paths (Fig. 2). This shifting by removing the source of irritation that is the injected solution from, or bringing it in closer proximity to, the cambium layer had a decided effect on the growth of the cambium and phloëm tissues.

THE WOUND TISSUE FORMED.

The character of the wound tissue depended on the toxicity of the injected solution. Quick killing was not followed by stimulation other than the formation of normal wound tissue (callus) to cover the wound. Fig. 2 shows an instance of this. The section was cut from a branch of a 9-year-old tree, which had been injected in May with meta cresol 1-1000 G.M., the branch having been cut in October. Callus had formed on both sides of the path of the injected chemical. The photograph shows one side of this path which could be distinguished readily by a stain (*s*). The callus which had formed is as normal as though the tree had been cut by a knife; the wound cambium or bark cambium (*bc*) surrounds the newly formed tissues; the groups of bast-fiber (*bf*), the protective cells, have the formation one sees in a one-year-old twig; the phloëm (*p*), cambium layer (*c*) and xylem (*x*) are normal.

There are transitions from such normal wound tissue as that just described, which are caused by the varying toxicity of the injected

chemicals. They exhibit more and more abnormality as wound tissue, until the sections show not wound tissue but stimulated growth of cells.

Fig. 3 shows a wound tissue formation in a ten-year-old tree which had been injected in June and July with nitro phenol-para 1-500 G.M. and felled in November. The path of the main stream is shown on the right-hand side of the photograph. The cells had been killed and a callus had formed which was not as well developed as the callus shown in Fig. 2. On the right-hand side the dark streak in the xylem shows the path of the solution. In this region the solution must have been much diluted as compared with the main stream. Here the phloëm, cambium layer (*c*) and year ring of wood are normal, with the exception of the xylem cells in the immediate neighborhood of the path of the solution. Somewhat left of the center a stimulating effect is seen in an abnormally placed group of xylem cells in the midst of the phloëm.

Questionable wound tissue is seen in Figs. 4 and 6. The xylem formation can be regarded as irregularly formed year rings of wood. In this case the growth of the rings of wood has been stimulated, for the normal ring of xylem had nearly completed its growth before injection began. Or the extra growth of xylem cells on the far side, as regards the cambium region, of the bast-fiber groups can be regarded as a form of wound tissue (callus). The premature bark, which always appears with the abnormal xylem, can be regarded as part of a wound tissue. Fig. 8 gives a detail showing the suggestive arrangement of the protective cells, stone cells, and bast-fibers, in relation to this extraordinary xylem.

INHIBITORY EFFECT ON THE GROWTH OF THE CAMBIUM.

This was a phenomenon common to the injected trees. Fig. 4 shows a section which had been cut from a sixteen-year-old tree, injected with lithium carbonate 1-500 G.M. in July, August, September and October and felled in November. The section shows the relation between the path of the injected alkali (*s*), the cambium layer region (*c*), the irregular growth of xylem cells (*x*) and the irregular growth of bark cambium (*bc*). Fig. 5 shows the enlarge-

ment of a portion of Fig. 4. In the center of the photograph in the region of the cambium layer are cambium cells which have changed into xylem. It shows also that the phloëm cells are changed into xylem by division and by thickening of the walls. A group of xylem cells has started growth on the far side of a group of bast-fibers. Fig. 6 shows a section from the same tree, in which this situation, exhibited in Fig. 4, is more pronounced. Fig. 7 shows an enlargement of the cambium region (*c*). Here it can be seen that the rows of bast-fibers are surrounded by xylem.

The inhibitory effect on the cambium was transitory. In time a new cambium layer formed, arising from phloëm cells. It separated the irregularly formed xylem from the phloëm. It formed a wavy row of cells, but its growth was normal.

PREMATURE FORMATION OF CORK.

Correlated with the abnormal growth of xylem in the phloëm region was a premature formation of cork. This was formed in so striking a manner that the path of an injected solution could be traced on a tree by the raised lumps of cork extending up and down the tree trunks. This cork formation was not in a continuous ridge of tissue, but appeared in irregularly shaped lumps. So certainly was its formation connected with a disturbance of the cambium and phloëm tissues, that one could tell by a cursory glance at a smooth barked tree at what points the above mentioned tissues were deranged. In the case of the lithium-injected trees, the spectroscope showed the presence of lithium in the cork, showing a connection between the abnormal tissues and the foreign chemical injected into the tree. Fig. 6 shows that the formation of this cork is due to an unusual development of bark cambium from phloëm cells.

XYLEM DEVELOPED FROM PHLOËM TISSUE.

The xylem which had formed by division of the phloëm cells, and often appeared to be a form of wound tissue, has been partially described. Fig. 8 shows the development of such xylem. The group of stone cells partially surrounded by xylem was part of a row of such cells. Rows of stone cells often were found in the

phloëm region in the injected trees. It can be seen that there is a close relation between the protective cells, i. e., stone cells and bast-fibers, and the xylem which is formed by cell-division of phloëm cells. Whether any of these groups of protective cells formed after injection future experiment will show. It seems probable that they did, as according to Moeller (2) such a formation can occur in the normal growth of the chestnut, *Castanea vesca*.

An irregular growth of the year ring of wood is shown in Fig. 9, which is a section cut from a fourteen-year-old tree injected May, June and July with nitro phenol-para 1-1000 G.M. and felled in November. The section shows the year ring of wood only. The formation of xylem on the far side of the bast-fibers is shown in a more pronounced form than in the previously discussed sections. No phloëm cells are in this section except bast-fiber and stone cells. The cells on the near side of the bast-fiber groups have been converted into xylem by a lignification of their walls.

Xylem also was formed by the thickening and lignification of phloëm cells without cell division. The section shown in Fig. 10 was cut from a fourteen-year-old tree, which had been injected in June and July with picric acid 1-1000 G.M. and felled in November. The cells of the dark-stained groups in the phloëm region have lignified cell walls and the shape of phloëm cells. This process of lignification is uninfluenced by the proximity of bast-fiber groups. No vessels are formed. It can be seen that the old phloëm rings are capable of change, for here the nine-year-old ring shows lignified cells. Fig. 11, shows a portion of Fig. 10, enlarged. In Fig. 11, a stone cell has been surrounded by xylem cells of abnormal shapes. The pits in the cell walls can be seen.

DISCUSSION.

The investigations of Schilberszky (3) showed that secondary extra-fascicular vascular bundles could be formed on the outer side of the bast-fiber cells in pea and bean seedlings. He found this new tissue developed by the division of the cells of the starch sheath or endodermis. The starch sheath acted as a cambium layer, producing phloëm cells on its outer side and xylem cells on its inner

side. He produced this result by cutting away a part of the seedlings' stems, when they were actively growing.

Schilberszky's result, obtained by cutting, was produced by the injections, when the solutions were toxic enough to kill or to retard the activities of the fibro-vascular bundles and the cambium layer. But the forms assumed by the deranged tissues, resulting from the injections, were not as definite as those obtained by the knife. This was probably for the reason that the toxic solutions did not usually make definite wounds, but killed only groups of cells or single cells, while other groups were simply retarded in growth or even stimulated. Also the shock from the incision was a single one, while in the case of the injections the source of irritation was present for weeks or months.

As stated before, Schilberszky found the extra-fascicular vascular bundles developed from the starch sheath, which is situated just outside the bast-fiber cells in the case of the pea and bean seedlings. This row of cells produced both phloëm and xylem tissue.

In the case of the injections the result cannot be attributed to the stimulation of a single tissue but rather of three: phloëm, cambium and xylem. So far the examination of the trees has not shown a development of new extra-phloëm cells. The xylem cells found in the phloëm region appear to have been produced by cell division of the phloëm cells followed by lignification, or by a lignification of the original phloëm cell walls. The isolated groups of xylem usually commenced to develop just behind the groups of bast-fiber, and this might indicate that the cells there were meristematic or cells which retained unusual regenerative powers. It has not been known hitherto that they were meristematic cells. It seems more probable from the way in which the groups of xylem cells increase, that these phloëm cells first respond to stimulation because of their position behind the bast-fiber groups. Osmosis in the case of the bast-fibers must be extremely slow. Therefore, an irritant coming from the direction of the injected chemical would strike the cells situated just back of the bast-fibers from two sides at once. Those phloëm cells, exposed directly and from one direction, would respond more slowly by a lignification of their cell walls.

All the cells of the phloëm are capable of change with the exception of the stone cells, the bast-fibers and their accompanying cells containing calcium oxalate crystals. Not only can the cells respond which are in the recently formed rings of phloëm, but those in the rings eight and nine years old. From the phloëm cells are formed bark cambium, cambium and xylem.

SUMMARY.

So far an examination of chestnut trees, injected with chemicals in solution, shows that the reaction in the trunks and branches, as evidenced by abnormal tissues, was alike in kind, but varied in intensity.

With increased distance from a point of injection the tissues became more normal and the area of disturbance decreased. All stages of reaction could be seen in a tree: death, inhibited growth, stimulated growth and no reaction.

The regions of response were the phloëm, cambium, and xylem.

The Phloëm.—The most remarkable response from a histological standpoint, was that given by the phloëm. Xylem cells formed in the midst of the phloëm region. This xylem was formed by division of the phloëm cells with subsequent lignification of the walls, or by lignification of the phloëm cell walls without division. All the cells in the phloëm were capable of change, except the stone cells, the bast-fibers and those cells containing calcium oxalate crystals, which accompany the bast-fibers.

The phloëm cells changed into bark cambium, cambium and xylem cells.

Cork formed prematurely, due to an unusual development of bark cambium in the phloëm region.

The Cambium.—The cambium layer often disappeared. Its cells were changed into xylem.

The Xylem.—The xylem responded by increased formation of thylloses and thickening of cell walls.

Wound Tissue.—Wound tissue formed which varied from normal to abnormal according to the toxicity of the solution injected.

BOTANICAL LABORATORY,
UNIVERSITY OF PENNSYLVANIA,
April 17, 1916.

LITERATURE CITED.

1. RUMBOLD, CAROLINE. Methods of Injecting Trees. *Phytopathology*, 5: 225-229. 1915.
2. MOELLER, JOSEPH. Anatomie der Baumrinden, p. 68. Berlin, 1882.
3. SCHILBERSZKY, KARL. Künstlich hervorgerufene Bildung sekundärer (extra faszikulärer) Gefässbündel bei Dikotyledonen. *Ber. d. D. bot. Ges.*, 10: 424. 1892.

EXPLANATION OF PLATES.

FIG. 1. Unstained free-hand section. Section cut from a tree eight years old, injected with methylene blue during the latter part of April. The tree was felled in October. Dilution of methylene blue solution was 1 gram to 4 liters of water.

FIG. 2. Auerbach stain. Section cut from a tree nine years old injected in May with meta-cresol 1-1000 G.M. The branch was cut from the tree in October.

FIG. 3. Delafield's hæmatoxylin. Section cut from a ten-year-old tree injected June and July with nitrophenol-para 1-500 G.M. Tree felled in November.

FIG. 4. Auerbach stain. Section cut from sixteen-year-old tree injected with lithium carbonate 1-500 G.M. in July, August, September and October. Tree felled in November.

FIG. 5. An enlargement of a part of Fig. 4.

FIG. 6. Auerbach stain. Section cut from the tree shown in Figs. 4 and 5.

FIG. 7. An enlargement of a part of Fig. 6.

FIG. 8. An enlargement of a part of Fig. 7.

FIG. 9. Heidenheim's iron alum hæmatoxylose. Section cut from a fourteen-year-old tree, which was injected May, June and July with nitrophenol-para 1-1000 G.M. Tree felled in November.

FIG. 10. Auerbach stain. Section cut from a fourteen-year-old tree which was injected in June and July with picric acid 1-1000 G.M. Tree felled in November.

FIG. 11. An enlargement of a part of Fig. 10.